



Opinion

On the “struggle between chemistry and biology during aging” – implications for DNA repair, apoptosis and proteolysis, and a novel route of intervention

Alan R. Hipkiss

*Division of Biomolecular Sciences, Henriette-Raphael House, GKT School of Biomedical Sciences, King's College London, Guy's Campus, London Bridge, London SE1 1UL, UK
(email: alan.hipkiss@kcl.ac.uk; fax: +44-207-8486399)*

Received 31 October 2000; accepted in revised form 15 December 2000

Key words: aberrant proteins, ageing, caspase, deamidation, DNA repair, isoaspartate, proteolysis

Abstract

The possible effects of specific spontaneous changes in protein chemistry on age-related homeostatic dysfunction are discussed. Spontaneous racemization and isomerization of aspartic acid and deamidation of asparagine to four possible forms of aspartic acid in caspases and their substrates could profoundly alter apoptotic activity. Deamidation of asparagine residues at critically important sites of DNA glycosylases could compromise base excision repair activity. Furthermore, as oxidative damage may enhance asparagine/aspartate instability in proteins, and erroneously-synthesized proteins show increased susceptibility to oxidative attack, it is beginning to appear that the aberrant protein forms that accumulate during ageing are possibly interrelated. The role of cell growth rates in controlling constitutive proteolytic elimination of various forms of aberrant polypeptides is then discussed. Finally, it is pointed out that three recently described agents that delay senescence in cultured cells (aminoguanidine, N-t-butylhydroxylamine and kinetin) resemble carnosine in that they are also likely to react with glycoxidised proteins, as well as possess anti-oxidant activity. These observations suggest that pluripotency may be a necessary pre-requisite for effective anti-ageing activity.

John Baynes' (2000) review on “the struggle between chemistry and biology during aging” together with those by Ward (2000) and Soti and Csermely (2000) have provoked the following questions on protein ageing. (i) Could amino acid residue instability directly affect homeostatic activities and thereby promote the ageing phenotype? (ii) Do developmentally-related changes in proteolytic activity contribute to the ageing phenotype? (iii) How may anti-ageing agents suppress the ageing phenotype?

Intrinsic amino acid instability, apoptosis, DNA repair and ageing

Baynes (2000) and Soti and Csermely (2000) discuss age-related deamidation of asparagine residues in

protein, a completely spontaneous process that yields any one of four products, L-aspartic acid, L-iso-aspartic acid, D-aspartic acid and D-iso-aspartic acid. Aspartic acid residues are also unstable (though less so than asparagine), they spontaneously racemize and isomerize to D-aspartic, D-iso-aspartic and L-iso-aspartic forms (Fujii et al. 1994, 1996). Recent studies suggest that oxidative conditions increase protein asparagine/aspartate instability (Ingrosso et al. 2000), and erroneously-synthesized proteins show increased susceptibility to oxidative damage (Dukan et al. 2000). Such observations provide not entirely unexpected links between various mechanistic ageing theories.

Formation of either D or isomeric forms of aspartic acid is important biologically because a repair

process has evolved, protein iso-aspartate methyltransferase (PIMT), that specifically methylates the free α -carboxyl group of D or iso-aspartic acid to promote formation of a succinimide intermediate which may rearrange to the normal form of aspartic acid (Kim et al. 1997). Complete repair to asparagine residues does not occur. 'Knock out' mice, deficient in PIMT activity, show decreased lifespan, neurological dysfunction and accumulate aberrant protein in their brains (Kim et al. 1997; Yamamoto et al. 1998). It appears that the resultant β -peptide bond, formed between iso-aspartate and the next amino acid residue, is resistant to proteolytic attack.

I suggest that the intrinsic instability of protein aspartic acid and asparagine residues could directly affect apoptosis and DNA repair and thereby contribute to age-related dysfunction and disease. Failure of apoptosis permits survival and proliferation of transformed cells increasing the likelihood of tumour development and cancer. In contrast, excessive or inappropriate apoptosis may contribute to neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease and motor neurone disease (amyotrophic lateral sclerosis). Apoptosis requires the function of a number of specific proteases called caspases in which aspartic acid residues are important. Caspases cleave at aspartic acid residues in their substrates, e.g., pro-caspases. Aspartic acid residues are also present at the active site of caspases and participate directly in peptide bond cleavage (Nicotera 2000). It is conceivable, therefore, that the intrinsic instability of asparagine and aspartic acid residues in caspases and their substrates could contribute to general age-related apoptotic dysfunction. First, asparagine deamidation to aspartic acid in any protein could create illegitimate aspartic acid cleavage sites in inappropriate polypeptides and sequester caspases from their proper substrates and function. Secondly, isomerization and racemization of aspartic acid residues to L or D-iso-aspartic acid or D-aspartic acid residues at either substrate cleavage site or at the enzyme's active site could again compromise function. Thirdly, asparagine deamidation would eliminate enzymically-mediated protein N-glycosylation sites which could also affect apoptosis (Kukuruzinska and Lennon 1998). More specifically, Yu et al. (2000) have recently shown that aspartic acid mutations in presenilins (caspases?) influence control of amyloid peptide precursor protein processing, amyloid peptide accumulation and Alzheimer's disease (Roher et al. 1993; Orpiszewski et al. 2000).

DNA repair is another homeostatic activity reported to show age-related dysfunction. Ageing is often characterized by increased oxidative DNA damage as shown by increased 8-oxoguanine content in nuclear and mitochondrial DNA, sometimes accompanied by a decline in base-excision repair activity (Hirano et al. 1995; Atamna et al. 2000a). Mutation in the gene coding for the DNA repair enzyme 8-oxoguanine DNA glycosylase (hOGG1) has been found in a strain of senescence accelerated mice (SAM) (Choi, et al. 1999). The resultant thermolabile protein has low DNA glycosylase activity and thus appears, at least partly, to be responsible for the accumulation of DNA damage (Hosokawa et al. 2000) and the premature ageing phenotype. The hOGG1 protein possesses a triplet of asparagine residues (149–150–151) required for the enzyme's interaction with DNA (Bruner et al. 2000). Furthermore Hardeland et al. (2000) have recently shown that a mutation in human thymine DNA glycosylase that results in substitution of an asparagine residue by an alanine at the active site of the enzyme compromises base-excision activity. It is not inconceivable that spontaneous loss of amide functions and/or generation of the various forms of aspartic acid residues at any one or more of hOGG1 asparagine triplet (149–151), or at position 140 of thymine DNA glycosylase, could affect DNA composition by compromising base-excision repair and eventually produce the mutator phenotype. Hence genomic changes that accompany ageing may be consequences of age-related epigenetic effects, at least in part.

Protein synthesis rates, proteolysis of aberrant protein and ageing

One determinant of whether changes in asparagine and aspartic acid residues occur in the hOGG1 protein molecule, presenilins, caspases and their substrates is the rate at which these proteins are replaced during normal turnover. Hence as protein replacement slows during normal ageing (Ward 2000) the chances that changes in protein asparagine and aspartic acid chemistry occur and persist are increased. It is not known if PIMT activity generally declines with age but it may (Pelletier et al. 1996). However, it is known that protein asparagine content often decreases with age, iso-aspartic acid levels generally increase and the β -amyloid peptide that accumulates in Alzheimer's disease brain is enriched in iso-aspartic acid residues (Orpiszewski et al. 2000; Najbauer et al. 1996; Roher

et al. 1993). Such observations are consistent with the proposal that a decline in proteolytic activities contribute to the accumulation of proteins bearing the consequences of asparagine and aspartic acid instability.

Ward (2000) identifies the necessity for a close coupling between protein synthesis and breakdown during cell development and suggests that changes in certain proteasome activities are important in the ageing phenotype. However it should be pointed out that cellular ability to deal with aberrant proteins by degrading, binding or refolding them, may not be constant during development (Soti and Csermerly 2000) and accumulation of aberrant polypeptides is a frequently observed age-related phenomenon. There are many sources of aberrant proteins which include erroneous synthesis and a number of post-synthetic modifications additional to the asparagine/aspartic acid instability described above. Particularly important are glycation by reactive sugars and other aldehydes and ketones, and oxidative damage by oxygen free-radicals and related species. As recently highlighted by Grune (2000), much of the oxidatively-damaged protein is selectively degraded by the proteasome system, but highly oxidized proteins, particularly if cross-linked, seem to be resistant to proteolysis and may even inhibit proteasome function towards potentially degradable substrates. It follows that excess cellular proteolytic activity is important for long-term viability. Should, however, degradation of aberrant polypeptides become limited by insufficient proteolytic activity, rather than substrate availability, then aberrant proteins could accumulate and cross-link into undegradable and inhibitory forms.

It is still unclear how development-related changes in cellular proteolytic activity could contribute to ageing (Rattan 1996), despite many observations showing age-related decline in a variety of proteolytic activities in many cell types (Rattan 1996; Carmichael and Hipkiss 1989; Wharton and Hipkiss 1985). Cellular proteolytic activities can be divided into constitutive and inducible components and it is likely, though unproven at present, that the ratio between them may not remain constant throughout a cell's lifetime, especially where large changes in growth rates occur. For example in *Escherichia coli* constitutive proteolytic activity squares as growth rate doubles (Rosenberger et al. 1990) and large differences in growth rates are found at various stages in mammalian development. Hence should growth-rate also affect constitutive proteolytic activity in mammalian cells

(cytoplasmic or mitochondrial compartments), then cellular history could influence constitutive degradative ability towards aberrant proteins. This idea is supported by the observation that constitutively-expressed ubiquitin is synthesized co-translationally with a ribosomal protein (Findlay et al. 1989). The proposal that constitutive proteolysis rates vary logarithmically with growth rates may provide some insight into the non-linear relationship between the appearance of the aged phenotype and the passage of time following cessation of growth. However at present there is great uncertainty about the rates at which the proteases, proteasomes, and components of the ubiquitin system turn over as cell growth rates decline. It is possible that as growth rates decline, upon cell division each daughter cell is provided excess proteolytic activity. Eventually, however, depending on the rate of degradation of the proteases themselves, a new lower steady-state level of proteolytic activity is established. When constitutive proteolytic activity is insufficient, induction of stress proteins and proteases etc. then becomes necessary, although the threshold for such induction would fall as constitutive proteolytic activity similarly falls as growth rates decline. Any delay between accumulation of aberrant polypeptides and the induction of sufficient proteases and chaperone proteins to deal with them, especially in slowly growing cells, could allow sufficient time for the deleterious molecules to exert any toxic/lethal effects. Additionally, as the complete stress response includes the cessation in the synthesis of regular housekeeping proteins, the persistence of stress can result in cell death due to the failure to replace intrinsically unstable or short-lived proteins essential for cell survival.

Anti-ageing strategies

Caloric restriction (CR) is the only widely demonstrated method by which ageing and many related conditions can be delayed, but not prevented, although mechanisms of CR's anti-ageing actions are not entirely understood. CR clearly impacts on homeostatic function (Soti and Csermerly 2000) and increased protein turnover could contribute to its beneficial effects.

Recent studies on the effects of anti-ageing agents support the views expressed by Baynes (2000) that phenotypic changes in chemistry can influence the ageing processes. Aminoguanidine (Fujisawa et al.

1999) and N-t-butylhydroxylamine (Atamna et al. 2000b) can delay ageing in cultured fibroblasts. Some time ago carnosine was shown to have very similar effects on cultured cells (McFarland and Holliday 1994, 1999). The mechanisms of these anti-ageing actions are uncertain and anti-oxidant activity has been proposed (Fujisawa et al. 1999; Atamna et al. 2000b), but it should be pointed out that better anti-oxidants than carnosine, aminoguanidine or N-t-butylhydroxylamine do not delay senescence in cultured cells which suggests that other activities of these molecules might also be required for anti-ageing function.

Carnosine is a potential anti-glycating agent protecting proteins (Hipkiss 1998; Hipkiss et al. 1995, 1997; Hipkiss and Chana 1998) and cells (Hipkiss et al. 1998) against reactive aldehydes and AGEs. Recent studies show that carnosine can react with glycoxidised proteins most probably via polypeptide-bound carbonyl groups, (Brownson and Hipkiss 2000; Hipkiss and Brownson 2000). Conceivably, similar adducts, i.e., 'carnosinylated' proteins between polypeptide carbonyls (formed by oxidation or glycation) and carnosine could occur in cultured cells and *in vivo*. Therefore the rejuvenating and anti-ageing effects of carnosine could be explained via possible metabolic rather than mere prophylactic actions, should 'carnosinylated' of protein carbonyls alter polypeptide fate or reactivity. It is possible that aminoguanidine and N-t-butylhydroxylamine could react with glycoxidised proteins in cultured fibroblasts similarly to that proposed for carnosine (Hipkiss and Brownson 2000). Aminoguanidine is a recognized anti-glycating agent that reacts with AGEs (Liggins and Furth 1997), whilst hydroxylamines generally are readily reactive towards aldehydes and ketones. Such activities could, like carnosine, moderate age-related accumulation of protein carbonyls, perhaps preventing formation of protein-protein cross-links that can inhibit proteasome function (Frigeut and Szewda 1997). Kinetin (N-6-furfuryl-adenine) has also been shown to delay appearance of the senescence phenotype in cultured human cells (Rattan and Clark 1994) and fruit flies (Sharma et al. 1995), and it has recently been shown that this molecule too possesses both antioxidant (Olsen et al. 1999) and anti-glycating activities (Verbeke et al. 2000). This again supports the proposal that anti-ageing action, at least in cultured cells, is dependent on both anti-glycating and anti-oxidant activities.

Proteasome activity is vital to cell function (Grune 2000; Keller et al. 2000), and many age-

associated protein changes can affect proteasome function. Proteins cross-linked by lipid peroxidation product 4-hydroxynonenal are inhibitory to proteasomes (Frigeut and Szewda 1997), as are aberrant forms of ubiquitin synthesized as a result of age-related transcriptional inaccuracy (Lam et al. 2000). Indeed it is possible that inhibition of proteasome function by any means, e.g., accumulation of inhibitors, aggregates of proteins and fragments refractory to proteolytic attack such as amyloid peptides and polyglutamine proteins, or dysfunction of the ubiquitination system induced by glycation or oxidation (Takizawa et al. 1993), could cause cell cycle arrest (senescence?) and/or provoke cell death by apoptosis or necrosis (Keller et al. 2000; Petropoulos et al. 2000). Does the reactivity of carnosine, aminoguanidine, N-t-butylhydroxylamine, and perhaps kinetin, towards protein carbonyls preserve proteasome function and possibly explain their anti-ageing actions towards cultured cells (Holliday and McFarland 2000; Atamna et al. 2000b; Fujisawa et al. 1999) and *in vivo* (Yuneva et al. 2000)? Studies on the mechanisms of action of these potential anti-ageing agents may reveal new routes whereby some age-related changes in cell chemistry might be delayed before giving up the struggle and succumbing to the inevitability of homeostatic dysfunction.

References

- Atamna H, Cheung I and Ames BN (2000a) A method for detecting abasic sites in living cells: age dependent changes in base excision repair. *Proc Natl Acad Sci USA* 97: 686-691
- Atamna H, Paller-Martinez A and Ames BN (2000b) N-t-butylhydroxylamine, a hydrolysis product of α -phenyl-N-t-butylhydrazine, is more potent in delaying senescence in human lung fibroblasts. *J Biol Chem* 275: 6741-6748
- Baynes JW (2000) From life to death - the struggle between chemistry and biology during aging: the Maillard reaction as an amplifier of genomic change. *Biogerontology* 1: 235-246
- Brownson C and Hipkiss AR (2000) Carnosine reacts with a glycated protein. *Free Rad Biol Med* 28: 1564-1570
- Bruner SD, Norman DPG and Verdine GL (2000) Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* 403: 859-866
- Carmichael PL and Hipkiss AR (1989) Age-related changes in proteolysis of aberrant crystallin in bovine lens cell-free preparations. *Mech Ageing Devel* 50: 37-48
- Choi JY, Kim HS, Kang HK, Lee DW, Choi EM and Chung MH (1999) Thermolabile 8-hydroxyguanine DNA glycosylase with low activity in senescence accelerated mice due to a single-base mutation. *Free Rad Biol Med* 27: 848-854
- Dukan S, Farewell A, Ballesteros M, Taddei F, Radman M and Nystrom T (2000) Protein Oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci USA* 97: 5746-5749

- Findley D, Bartlett B and Varshavsky A (1989) The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosomal biogenesis. *Nature* 338: 394-401
- Fujisawa H, Nishikawa T, Zhu BH, Nishimura Y, Shimizu M, Kimoto M, Higuchi K and Hosokawa M (1999) Aminoguanidine supplementation delays the onset of senescence *in vitro* in dermal fibroblast-like cells from senescence-accelerated mice. *J Gerontol* 54: 276-282
- Friguet B and Szewda LI (1997) Inhibition of the multicatalytic protease (proteasome) by 4-hydroxynonenal cross-linked protein. *FEBS Lett* 405: 21-25
- Fujii N, Momose Y and Harada K (1996) Kinetic studies of racemization of aspartyl residues in model peptides of aA-crystallin. *Int J Peptide Protein Res* 48: 118-122
- Fujii N, Satoh K, Harada K and Ishibashi K (1994) Simultaneous stereoinversion and isomerization at specific aspartic acid residues in aA-crystallin from human lens. *J Biochem* 116: 663-669
- Grune T (2000) Oxidative stress, aging and the proteosomal system. *Biogerontology* 1: 31-40
- Hardeland U, Bentele M, Jiricny J and Schar P (2000) Separating substrate recognition from base hydrolysis in human thymine DNA glycosylase by mutational analysis. *J Biol Chem* 275: 33449-33456
- Hipkiss AR (1998) Carnosine, a protective anti-ageing peptide? *Int J Biochem Cell Biol* 30: 863-868
- Hipkiss AR and Brownson C (2000) Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? *Biogerontology* 1: 217-223
- Hipkiss AR and Chana H (1998) Carnosine protects proteins against methylglyoxal-mediated modifications. *Biochem Biophys Res Commun* 248: 28-32
- Hipkiss AR, Michaelis J and Syrris P (1995) Non-enzymic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent. *FEBS Lett* 371: 81-85
- Hipkiss AR, Preston JE, Himsworth DTJ, Worthington VC and Abbott NJ (1997) Protective effects of carnosine against malondialdehyde-induced toxicity towards cultured rat brain endothelial cells. *Neurosci Lett* 238: 135-138
- Hipkiss AR, Worthington VC, Himsworth DTM and Herwig W (1998) Protective effects of carnosine against protein modification mediated by malondialdehyde and hypochlorite. *Biochim Biophys Acta* 1380: 46-54
- Hirano T, Yamaguchi Y, Hirano H and Kasai H (1995) Age-associated change of 8-hydroxyguanine repair activity in cultured human fibroblasts. *Biochem Biophys Res Commun* 214: 1157-1162
- Holliday R and McFarland GA (2000) A role for carnosine in cellular maintenance. *Biochemistry (Moscow)* 65: 843-848
- Hosokawa M, Fujisawa H, Ax S, Zahn-Daimler G and Zahn RK (2000) Age-associated DNA damage is accelerated in the senescence accelerated mice. *Mech Ageing Devel* 118: 61-70
- Ingrasso D, D'Angelo S, di Carlo E et al. (2000) Increased methyl esterification of altered aspartyl residues in erythrocyte membrane proteins in response to oxidative stress. *Eur J Biochem* 267: 4397-4405
- Keller JN, Hanni KB and Markesbery WR (2000) Possible involvement of proteasome inhibition in aging: implications for oxidative stress. *Mech Ageing Devel* 113: 61-70
- Kim E, Lowenson JD, MacLaren DC, Clarke S and Young SG (1997) Deficiency of a protein repair enzyme results in the accumulation of altered proteins, retardation of growth and fatal seizures in mice. *Proc Natl Acad Sci USA* 94: 6132-6137
- Kukuruzinska MA and Lennon K (1998) Protein N-glycosylation: molecular genetics and functional significance. *Crit Rev Oral Biol Med* 9: 415-448
- Lam YA, Pickart CM, Alban A, Landon M, Jamieson C, Ramage R, Mayer RJ and Layfield R (2000) Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. *Proc Natl Acad Sci USA* 97: 9902-9906
- Liggins J and Furth AJ (1997) Role of protein-bound carbonyl groups in the formation of advanced glycosylation end products. *Biochim Biophys Acta* 1361: 123-130
- McFarland GA and Holliday R (1994) Retardation of the senescence of cultured human diploid fibroblasts by carnosine. *Exp Cell Res* 212: 167-175
- McFarland GA and Holliday R (1999) Further evidence for the rejuvenating effects of the dipeptide L-carnosine on cultured human diploid fibroblasts. *Exp Gerontol* 34: 35-45
- Naibauer J, Orpiszewski J and Aswad DW (1996) Molecular aging of tubulin: accumulation of isoaspartyl sites *in vitro* and *in vivo*. *Biochemistry* 35: 5183-5190
- Nicotera P (2000) Caspase requirement for neuronal apoptosis and neurodegeneration. *IUBMB Life* 49: 420-425
- Olsen, A, Siboska G, Clark BFC, and Rattan SIS (1999) N⁶-Furfuryladenine, kinetin, protects against Fenton reaction-mediated oxidative damage to DNA. *Biochem. Biophys Res Commun* 265: 499-502
- Orpiszewski J, Schormann N, Kluge-Beckerman B et al. (2000) Protein aging hypothesis of Alzheimer's disease. *FASEB J* 14: 1255-1263
- Pelletier J, Desrosiers RR and Beliveau R (1996) Age-related changes in carboxyl methylation of proteins in the kidney. *Mech Ageing Devel* 86: 115-135
- Petropoulos I, Conconi M, Wang X, Hoenel B, Bregger F, Milner Y and Friget B (2000) Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. *J Gerontol Biol Sci Med Sci* 55: B220-B227
- Rattan SIS (1996) Synthesis, modifications and turnover of proteins during aging. *Exp Gerontol* 31: 33-47
- Rattan SIS and Clark BFC (1994) Kinetin delays the onset of aging characteristics in human fibroblasts. *Biochem Biophys Res Commun* 201: 665-672
- Roher AE, Lowenson JD, Clarke S. et al. (1993) Structural alterations in the peptide backbone of β -amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J Biol Chem* 268: 3072-3083
- Rosenberger RS, Carr AJ and Hipkiss AR (1990) Regulation of breakdown of canavanine proteins in *Escherichia coli* by growth conditions in Lon⁺ and Lon⁻ cells. *FEMS Lett* 68: 19-25
- Sharma SP, Kaur P and Rattan SIS (1995) Plant growth hormone kinetin delays aging, prolongs the life-span and slows down development of the fruit-fly *Zapionus parvittiger*. *Biochem Biophys Res Commun* 216: 1067-1071
- Soti C and Csermely P (2000) Molecular chaperones and the aging process. *Biogerontology* 1: 225-233
- Takizawa N, Takada K and Ohkawa K (1993) Inhibitory effect of nonenzymic glycation on ubiquitination and ubiquitin-mediated degradation of lysozyme. *Biochem Biophys Res Commun* 192: 700-706
- Verbeke P, Siboska GE, Clark BFC and Rattan SIS (2000) Kinetin inhibits protein oxidation and glycoxidation *in vitro*. *Biochem Biophys Res Commun* 276: 1265-1270
- Ward WF (2000) The relentless effects of the aging process on protein turnover. *Biogerontology* 1: 195-199

- Wharton SA and Hipkiss AR (1985) Degradation of peptides and proteins of different sizes by homogenates of human MRC-5 lung fibroblasts: aged cells have a decreased ability to degrade shortened proteins. *FEBS Lett* 184: 249-253
- Yamamoto A, Takagi H, Kitsuoka D. et al. (1998). Deficiency in protein L-isoaspartyl methyltransferase results in a fatal progressive epilepsy. *J Neurosci* 18: 2063-2074
- Yu G, Chen F, Nishimura M, Steiner H, Tandon A, Kararai T, Arawaka S, Song Y-Q, Rogaeva E, Holmes E, Zhang DM, Milman P, Fraser P, Haas C and St. George-Hyslop PJ (2000) Mutation of conserved aspartates affects maturation of both aspartate mutant and endogenous presenilin 1 and presenilin 2 complexes. *J Biol Chem* 275: 27348-27353
- Yuneva MO, Bulygina ER, Gallant SC, Kramarenko GG, Stvolinsky SL, Semyonova ML and Boldyrev AA (2000) Effect of carnosine on age-induced changes in senescence-accelerated mice. *J Anti-Aging Med.* 2: 337-342